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PRODUCTION OF FEMALE OFFSPRING BY
VIRGIN FEMALES IN THE GREENHOUSE
WHITEFLY, *TRIALEURODES VAPO-
RARIORUM*, UNDER THE
INFLUENCE OF HIGH
TEMPERATURES¹

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VIRGIN females of the white fly, *Trialeurodes vaporariorum*, in America have been shown to produce eggs which normally hatch into haploid males, while fertilized eggs produce diploid females (Morrill, 1903; Stoll and Shull, 1919; Schrader, 1920). It was therefore a wholly unexpected result when some virgin females subjected to high temperature produced occasional females among the predominating males. Under normal temperature conditions in other experiments at the same time, virgin female white flies produced only males. The problem which immediately presented itself was to discover the manner of the production of these exceptional females. Parthenogenetic production of females by normally arrhenotokous virgin females under the influence of high temperatures seems never to have been reported. However, polyploidy has been produced in certain animals by exposing their eggs to either high or low temperatures (Just, 1929; Gross, 1932; Hasimota, 1933; Astaurov, 1936; Frolova, 1940; Griffiths, 1941; Fankhauser and Watson, 1942), and females in this case could result from a multiplication of chromosome numbers.

Parthenogenesis in the American white fly was first reported by Morrill (1903) who demonstrated that virgin females produced only males. This report was later contradicted by Hargreaves (1915) and Williams (1917), who both found that virgin females of an English race of

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T. vaporariorum gave rise only to females. Stoll and Shull (1919) confirmed through their experiments the conclusion that virgin females of the American race yielded only males and that mated females often produced many more females than males. A cytological analysis of the American race by Schrader (1920) indicated that females arose from fertilized eggs and males from unfertilized eggs; females possessed twenty-two chromosomes and males were found to have eleven.

Schrader (1926) believed that there existed two distinct races of *T. vaporariorum*. Although the two races were morphologically similar, the American race exhibited arrhenotokous, the English race thelytokous parthenogenesis. This could account for the discrepancies between the conclusions of American and English workers. Thomsen (1927) described both arrhenotokous and thelytokous races from Denmark. In eggs of the thelytokous race 22 chromosomes are retained, while in the arrhenotokous race there is a reduction division, and eggs with eleven chromosomes are produced. Hence, only females were found in the thelytokous race, and both males and females in the arrhenotokous race.

CONTROL EXPERIMENTS

In order to determine whether the virgin females were of the arrhenotokous race, virgin white-fly females were placed on protected *Calendula* plants, and permitted to produce offspring at 20° C. The flies used in these experiments were collected on tomato plants in the University of Michigan Botanical Gardens in October, 1939.

Virgin females were collected by the following method. Leaves bearing mature pupae were picked from stock plants and placed on moist blotting paper. After two hours, the females that had emerged were collected; they were, of course, virgin. It was found that sunlight increased the rate of emergence, and by thus controlling the amount of light, virgins could be obtained at the proper time.

The control experiments were kept at room temperature which was about 20° C. Throughout the entire winter, experiments were performed beginning as soon as the weather became cool enough to permit the laboratory room to remain below 22° C. During the fall and spring months those experiments which were subjected to room temperatures above 23° C. were discarded. The virgin females were left on the plants for several weeks and removed before their offspring emerged. In forty-four experiments, a total of 410 virgin females produced 2,440 males and no females. This is in agreement with the findings of Morrill (1903), Stoll and Shull (1919) and Schrader (1920) that virgin female white flies produce only males by parthenogenesis.

HIGH-TEMPERATURE EXPERIMENTS

Virgin females were subjected to temperatures ranging from 37° C. to 45° C. for various lengths of time. This was done in an attempt to find the optimum temperature and the length of treatment needed to induce the virgin females to produce females. Both single and repeated heat shocks were employed.

Since both the insects and plants were placed at elevated temperatures, an illuminated chamber was used. This aided the plant in surviving abnormal temperatures; if the plant died while the larvae were young, all these offspring were lost. The high-temperature chamber was thirty-two inches long, twenty inches high and twenty inches deep. On each of the long sides there was an electric heater. At one end there was a double glass-plate window, outside of which were suspended two 150-watt clear-glass bulbs. Water was circulated between the glass panes to remove the heat produced by the electric bulbs.

There were twenty-five successful experiments in which virgin females were induced to produce females under the influence of high temperatures (Table 1). In these experiments, 161 virgin white flies produced 2,845 males and

TABLE 1

EXPERIMENTS IN WHICH VIRGIN FEMALES SUBJECTED TO HIGH TEMPERATURES WERE INDUCED TO PRODUCE EXCEPTIONAL FEMALES

Exp.	No. of virgin females	Temp.	No. heat treatments	Total time in hours	Offspring	
					Males	Females
		° C.				
V3h	9	38	1	5	8	1
V4h	7	39	8	8	140	24
V5h	8	38	2	28	90	18
XX2	9	38	4	19	151	32
XX5	10	38	4	18	205	10
XX6	11	38	4	18	69	2
XX7	5	38	2	28	82	1
V11X1	4	38	8	16	40	1
V14X1	7	39	4	45	48	35
Vtxh	4	40	3	36	120	1
V22X1	5	43	8	19	142	1
V51X1	9	39	2	12	253	1
V52h	9	38	3	19	145	17
V53h	7	39	2	13	186	1
V54h	7	39	2	12	114	16
V56h	6	38	1	5	75	42
V66h	3	38	4	45	199	1
V67h	2	39	5	61	75	1
V83h	6	40	1	8	9	57
V98h	7	40	2	23	0	2
V129h	8	40	1	6	179	1
V171h	4	42	4	17	225	1
V175h	5	43	3	12	148	1
V176h	7	38	2	19	124	1
V182h	6	39	3	22	18	33
Total	161				2,845	299

299 exceptional females. The temperatures ranged from 38° C. to 43° C., and the length of heat treatments varied from one five-hour treatment at 38° C. to five treatments totaling sixty-one hours at 39° C. There were four experiments in which only one high-temperature exposure was necessary to induce virgin females to produce females. The majority of the successful heat experiments involved more than two heat treatments. There was wide variation in the percentage of exceptional females in the progeny from 0.4 per cent. (253 males and one female) in V51X1 to 100 per cent. (no males and two females) in V98h.

Besides the successful attempts, there were sixty-six experiments in which virgin females were subjected to high temperatures and failed to produce exceptional females. A total of five hundred virgin females were placed at high temperatures, and produced 6,932 males and no females. There was no obvious difference between the treatment of the flies in these unsuccessful experi-

ments and that of the virgin females which did produce daughters. Indeed, in a number of pairs or groups of experiments in which the temperature and the length of treatment were identical and applied simultaneously the results were different, for in some the virgin females produced exceptional females, while in the other or others involving exactly the same conditions only males were produced. For example, experiment XX2 (Table 1) was accompanied by another which was given precisely the same treatment; the recorded parents (XX2) produced 151 males and 32 females, while in the unsuccessful one the virgin females produced 101 males and no females.

In a number of the experiments, the virgin females were permitted to lay eggs at room temperature, before they were transferred to another plant and heat shocked. This was done to test the females before they were subjected to high temperatures. In one experiment ten virgin females were allowed to lay eggs for several days. The virgin females were then removed and placed on V14X1 which was subjected to four heat treatments at 39° C. for a total of 45 hours (Table 1). Three males and no females were produced before treatment, but forty-eight males and thirty-five females after treatment. In another experiment twelve virgin females produced (in five days) sixty males and no females; the virgins were then transferred to V22X1 (Table 1) and heat treated a total of nineteen hours at 43° C., after which they produced 142 males and one female. These groups of families present some evidence that the virgin females were male-producing before being subjected to high temperatures.

PUPAE AND EGGS SUBJECTED TO HIGH TEMPERATURES

After adult virgin females had been induced to produce females under the influence of high temperatures, the pupae and eggs were then subjected to the same treatment to determine whether or not exposure of these

stages to the high temperatures would influence the adult female. Pupae were treated at temperatures varying from 38° C. to 47° C. The virgin females which emerged from these pupae were then placed on protected plants and the offspring examined for exceptional females. None were found in nine experiments in which a total of sixty-seven virgin females produced 618 males and no females.

Eggs which were laid by virgin females were exposed to temperatures of 38° C., 39° C. and 40° C. with the hope of producing exceptional females. In these experiments 124 males and no females were produced.

From this evidence it appears that high temperatures applied to the pupa do not cause it, as a virgin adult, to produce females; nor does exposure of unfertilized eggs to high temperatures induce them to develop into females.

VIRGIN FEMALES SUBJECTED TO LOW TEMPERATURES

An attempt was made to duplicate the heat-shock results with low temperatures. Virgin females were placed on plants and kept at room temperature for a day to feed and obtain strength to endure the cold. They were then placed at low temperatures and later tested for thelytoky. The low temperature chamber was cooled by brine and maintained temperatures ranging from 4° C. to 7° C. In six experiments a total of forty-two virgins were subjected to low temperatures for 18 to 98 hours and produced 650 males and no females. The low temperatures did not induce the virgin females to produce females.

VIRGIN FEMALES SUBJECTED TO BOTH LOW AND HIGH TEMPERATURES

Virgin females were first exposed at low temperatures and then transferred to high temperatures with the hope that the sudden change and the greater range of temperature would induce the virgin females to produce some exceptional females. In the majority of the experiments

the virgin females were placed at temperatures near 5° C. for from 11 to 33 hours, and then removed from the cold chamber and placed at temperatures about 40° C. In four of these experiments no females were produced, but in the fifth one, V71CH, one or more of the ten virgin females were induced to produce females (Table 2). In this latter

TABLE 2
VIRGIN FEMALES SUBJECTED TO LOW TEMPERATURES FOLLOWED BY
HIGH TEMPERATURES

Exp.	Virgin parents	Temp.	No. of treat- ments	Total time	Temp.	No.	Total time	Offspring	
								Males	Females
		° C.		hrs.	° C.		hrs.		
V32X1	13	4	3	33	40	3	68	124	0
V34X1	7	5	1	12	40	1	13	24	0
V45X1	6	5	1	11	40	1	12	173	0
V46X1	10	4	1	12	38	1	12	247	0
V71CH	10	6	3	87	40	3	38	80	16
Total	46							653	16

experiment the flies were left at 6° C. for 87 hours. This may be the reason why the virgin females were induced to produce females. However, as shown above, high temperatures alone sometimes induce the virgin females to produce females, and thus the low temperature may have had no influence.

IS THE HIGH-TEMPERATURE EFFECT TRANSMITTED?

Virgin females were obtained from exceptional females and were first tested for thelytoky at room temperature. These virgins were later exposed to high temperatures. In one series of experiments, five virgin females were obtained from progeny produced by the exceptional female in V11X1 (Table 1). At room temperature these virgins produced 116 males and no females. Four of the virgin females (one lost on transfer) were then subjected to high temperatures and produced 120 males and one female (Vtxh, Table 1).

Out of the thirty-five exceptional females produced by the influence of high temperatures in V14X1 (Table 1), four were known to be virgins and were tested by being

isolated on a plant; these virgins produced 123 males and no females. From the other thirty-one females that had mated with their brothers, virgin females were obtained. These virgin females produced only males at room temperature, and after being exposed to high temperatures continued to produce males.

From the above results it appears that exceptional females and their progeny behave like stock females with regard to parthenogenesis. Virgin females produced only males at room temperature, and when subjected to high temperatures the virgin females sometimes produced females along with the males. Thus, one can conclude that the high temperature effect, in which the virgin female is induced to produce females, is not transmitted from one generation to another.

COLCHICUM EXPERIMENTS

Another method of inducing polyploidy has been the influence of the alkaloid colchicine on chromosome separation (Blakeslee and Avery, 1937; Nebel and Ruttle, 1938). Since colchicine is water-soluble, the drug could not penetrate the wax-covered body wall if the insects were to be dipped in a solution of colchicine. Microinjection apparatus was not available, so a crude method was employed. The virgin females were placed on *Colchicum autumnale*, the plant from which colchicine is extracted, and allowed to feed. Although colchicine is extracted from the seeds of the plant, there is enough present in the leaves to poison cattle (Hegi, 1926). In feeding upon the plant sap, colchicine was probably taken into the alimentary canal, and there was a possibility that some of the colchicine could diffuse and affect the eggs.

The virgin females were left on the *Colchicum* plant for periods ranging from two to twenty-four hours, and removed to young *Calendula* plants. Although the flies were observed to go through the feeding motions, most of the females appeared to be starved after twenty-four hours. The *Colchicum* plant had hard, thick leaves (like

those of the rubber plant) and this may have prevented the stylet from reaching the leaf sap. The *Colchicum* plants were grown under large lantern globes to prevent stray white flies from getting on the plant before the virgin females were placed on them. The insects were placed on young leaves, for these leaves were softer and the flies would have less difficulty in feeding.

Although twenty-one experiments were started, only in eleven were offspring produced. Exceptional females were found in two of these. In experiment C1 two exceptional females were produced, and one female was counted in C6 (Table 3). In all, a total of sixty-six virgin

TABLE 3
VIRGIN FEMALES ALLOWED TO FEED ON *Colchicum autumnale* AND THEN
TRANSFERRED TO A CALENDULA PLANT

Exp.	Virgin females used as parents	Length of treatment	Offspring	
			Males	Females
		<i>hrs.</i>		
C1	10	24	18	2
C2	3	20	1	0
C3	6	18	43	0
C4	2	22	7	0
C5	5	24	115	0
C6	5	24	100	1
C7	10	24	61	0
C9	6	22	141	0
C11	5	23	46	0
C16	4	24	37	0
C21	10	24	64	0
Total	66		633	3

females produced 633 males and three females (Table 3). The exceptional females appeared to be similar to the stock (control) females.

CYTOLOGY

Since it was suspected that the production of females by virgin females was the result of polyploidy, chromosome counts were made of adults, pupae, larvae and eggs of both control and heat-produced females. The white-fly material was first dipped into Carnoy's No. 2 solution to remove the wax and then fixed in Navashin's fluid. Heidenhain's iron hematoxylin was used as the stain.

The chromosome number of all the stock females

counted was twenty-two. The chromosome number of five exceptional females was also determined and there was no difference. The control and exceptional pupae were found to have eleven chromosomes in the males and twenty-two in the females. All the stock embryos examined for chromosome number had either eleven or twenty-two chromosomes. At the metaphase of the first division in the maturation of both a control egg and an egg produced by an exceptional female, there were eleven tetrads. The eggs at the end of the second division of the maturation process possessed eleven chromosomes, which is in agreement with the work of Schrader (1920) and Thomsen (1927) on white-fly cytology.

DISCUSSION

The production of exceptional females by subjecting the virgin females to high temperatures can best be explained by assuming that the maturation process is altered and the diploid number of chromosomes is retained by the egg. Under normal conditions the virgin white fly produces only males, and mated females produce both males and females; a fertilized egg results in a female. The cytological examination demonstrated that the males have eleven chromosomes while the females possess twenty-two. An egg laid by a virgin female has eleven chromosomes at the end of the maturation division and is destined to become a male. However, under the influence of high temperatures the virgin females are induced to produce some exceptional females. This may be due to the retention of the diploid number of chromosomes, for all the chromosome counts of the exceptional females indicated the presence of twenty-two chromosomes.

In the silkworm studies (Astaurov, 1936; Frolova, 1940) and the newt experiments (Griffiths, 1941; Fankhauser and Watson, 1942) in which polyploidy was induced in eggs by extremes of temperature, the eggs were outside the body of the female. The eggs were in some

phase of the maturation process when the low or high temperature was applied. In the case of the white flies the eggs were subjected to the high temperature while still in the female. In the multiple-heat-shock series, it was possible that some of the eggs were in one of the maturation divisions when placed in the high-temperature chamber. This can account for the experiments in which only one or two exceptional females were produced. However, one can not use the theory of direct action of high temperatures on the maturation divisions in the case of experiment V83h (Table 1), in which a short heat treatment induced the virgin females to produce a comparatively large number of females (57 females and nine males). The white-fly female lays about three eggs per day. In experiment V83h the six virgins that were used as parents were subjected to 40° C. for eight hours. The maximum number of eggs that were in the process of maturation during the period of high temperature must have been about twenty. However, fifty-seven females were produced. One may assume that the high temperature affected the immature eggs in the ovaries of the virgin females. These eggs, when they have matured to the extent necessary to start the maturation process, may still retain the heat influence which results in the eggs retaining the diploid number of chromosomes.

The production of the exceptional females by virgin females occurred only in the experiments in which adult flies were exposed to high or low temperatures. The experiments in which pupae were heat-shocked and the virgin females emerging from them tested for thelytoky were few in number. However, in the nine experiments not one female was found among the 618 males produced. There were three experiments in which unfertilized eggs were subjected to high temperatures and failed to produce exceptional females. It would appear that the heat effect upon the eggs or germinal tissue of the females occurs only in the adult female.

The multiple heat shocks appeared to induce the virgin

females to produce females with greater ease than single-shock experiments, for in the successful experiments there were four single and twenty-one multiple-heat-shock experiments (84 per cent. multiple), while in the unsuccessful experiments there were twenty-one single and forty-five multiple (68.1 per cent. multiple). It would seem that several heat shocks in succession would be more likely to catch the virgin females in the proper stage for the heat to affect the eggs.

The existence of a thelytokous race of *T. vaporariorum* in Denmark has been reported by Thomsen (1927). Since there were many generations in which no male appeared, there must be some genetic control which maintains the diploid condition. The action of the high temperature may be the altering of a process or processes which result in the egg maintaining its diploid condition. The temperature change may have the same effect as a gene or genes in this case. The genetic processes in control of the maturation may be altered by the high temperatures, but the effect is not passed on to future generations, for this heat influence upon virgin females is not permanent or a fixed mutation, because the exceptional females and their progeny when tested behaved like normal white flies. Even the exceptional females when obtained as virgins and tested produced only males.

If a mutation had been discovered, it would have aided in determining exactly where in the maturation process the disruption takes place that results in the egg possessing a diploid number of chromosomes. One of the divisions may be suppressed, or the fusion of one of the polar bodies with the egg nucleus can produce a diploid egg. Frolova (1940) reported, in the study of the maturation of unfertilized eggs of *Bombyx mori* induced to develop by high temperatures, that the reduction division was eliminated, one polar body was formed, and the diploid chromosome number was retained in the pronucleus. This inhibition of the reduction division was due to the stretching of the spindle, which prevented the

rotation of the tetrads and the formation of an elimination plate.

The Colchicum experiments were not as successful as the high-temperature experiments in inducing virgin females to produce females. The three exceptional females produced by virgin females after feeding on the Colchicum plant were morphologically similar to the control females. These exceptional females were probably produced by the colchicine diffusing through the alimentary canal and affecting the eggs by means of the open circulatory system. Colchicine is well known for its ability to induce polyploidy by disrupting chromosome separation in animals and plants. Hence, these exceptional females add more evidence that females produced by virgins were due to polyploidy which probably resulted from faulty chromosome separation.

In this study of the influence of high temperature upon the production of females by virgin females, the problem of sex determination is encountered. As has been mentioned above, the would-be males are induced to become females under the influence of high temperature by a probable retention in the egg of the diploid number. From the results, not much can be said about the sex determination of this particular insect other than what has been written by other authors. No sex chromosomes can be distinguished. In the white fly, possession of a haploid or diploid number of chromosome is the feature which determines sex. Thus a diploid number of genes acts differently from a haploid set of genes in production of sex in this insect. It is evident that in doubling the chromosome number the processes that would have gone on to produce a male white fly are altered and a female results. The high temperature to which the virgin females were subjected does not take the place of an extra set of genes, but produces an influence on the maturation division, which results in the egg retaining its diploid condition.

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SUMMARY

Under normal temperature conditions (20° C.), the virgin white-fly female produces only males; females result from fertilized eggs. Virgin white-fly females, when subjected to temperatures about 38° C., were induced sometimes to produce exceptional females.

In forty-four control experiments, 410 virgin females produced 2,440 males and no females.

In a total of ninety-one experiments 661 virgin females were subjected to temperatures ranging from 37° C. to 45° C. In twenty-five of the experiments, 161 virgin females produced 299 exceptional females and 2,845 males. The other sixty-six experiments failed to produce females. Multiple heat treatments were more successful in production of exceptional females.

Low temperatures failed to induce virgin females to become thelytokous. Virgin females that had been exposed to high temperatures while in the pupal stage failed to produce females. Unfertilized eggs exposed to high temperatures developed only into males.

Exceptional females have the same diploid number of chromosomes, twenty-two, as the stock (control) females. Males have eleven chromosomes. Virgin exceptional females produced only males at room temperature; the heat effect is not passed on from generation to generation.

Virgin females were permitted to feed on *Colchicum autumnale*, which contains colchicine, and then tested for thelytoky. In two of the eleven experiments, three exceptional females were found.

The production of females by virgin females may be due to the retention of the diploid number of chromosomes in the egg under the influence of high temperature. The second polar body may fuse with the egg nucleus or

there could be a faulty separation of the chromosomes during one of the divisions in the maturation process.

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